

Green Infrastructure Stormwater Nitrogen BMP

SAMPLING PROTOCOL STANDARD OPERATING PROCEDURES

October 5, 2017 – Version 3.2

**Intersection of South Street and Pleasant Street
Barnstable, MA**

Contents:

1. OVERVIEW	2
Introduction	2
Sampling Program Background.....	2
Purpose of this Document	3
2. AUTO-SAMPLER PROGRAMMING.....	4
3. PRE-SAMPLING EVENT PROCEDURES.....	6
4. SAMPLING PROCEDURES	8
5. POST-SAMPLING AND SAMPLE PREPARATION PROCEDURES	11
6. ANNUAL PROGRAM STARTUP GUIDELINES.....	15
7. ROUTINE BI-ANNUAL MAINTENANCE GUIDELINES	16
8. CONTACT INFORMATION.....	21
9. TROUBLESHOOTING PROGRAMMING PROCEDURES	22

Attachments:

Attachment A: Bacteria Sampling SOPs

Attachment B: Sampling Checklists

Attachment C: Equipment Inventory



1. OVERVIEW

Introduction

The coastal embayments of Cape Cod have historically received excess nitrogen loadings, with a portion of nitrogen coming from stormwater runoff. Consequently, the Massachusetts Estuaries Project (MEP) developed total maximum daily load allocations (TMDLs) for many southern Massachusetts embayments including those in Cape Cod. To begin the process of reaching the TMDL goals, the Town of Barnstable partnered with the United States Environmental Protection Agency (EPA), WaterVision, LLC, and Comprehensive Environmental Inc. (CEI) to initiate a pilot project in Cape Cod in 2014 and demonstrate the effectiveness of nitrogen load-reducing stormwater BMPs. This project was designed to monitor and quantify the BMP performance for nitrogen removal.

Sampling Program Background

The purpose of the monitoring program for the Barnstable BMP is to quantify the nitrogen load-reduction performance of the subsurface gravel wetland. To quantify the effectiveness of the Barnstable subsurface gravel BMP, parameters including flow, total nitrogen (TN), total phosphorus (TP), and total suspended solids (TSS) will be gathered at the inlet and outlet of the BMP. These measurements will be analyzed to compare the percentage of nutrients and sediment entering and leaving the treatment system.

The above laboratory-analyzed sampling data will be supplemented through the use of data collected using automated sondes also located at the entrance and exit of the treatment system which regularly take readings at fixed intervals. Sondes will automatically measure dissolved oxygen (DO), temperature, conductivity, and pH which can then be matched to other data by reviewing the time and date of collection.

Optional bacteria sampling at both the inlet and outlet may also be performed by collecting manual grab-samples using the SOPs for bacteria collection provided in **Attachment A** for analysis by a laboratory chosen by the Town. Note that for Barnstable, a separate bacteria grab sample should be collected from within the trunk line baseflow as a “background” or baseline condition, for a total of 3 samples.



It is anticipated that data will be collected for 20 or more rain events or varying intensities and rainfall depths over a period of two to five years. Ultimately, successful reduction of nitrogen loadings, along with phosphorus and total suspended solids loadings, from the stormwater runoff entering Hyannis Harbor will promote further stormwater BMP development in Cape Cod to reach TMDL recommendations for nutrient removal.

Purpose of this Document

The following Standard Operating Procedures (SOPs) and checklists in **Attachment B** contained within this document are intended to provide easy to follow instructions on how to perform ongoing sampling events, including what to do before a storm begins, how to sample during a storm, periodic maintenance required, along with other pertinent information that may be applicable to volunteer personnel. Summary sheets on equipment and pertinent information is located in **Attachment C**.



2. AUTO-SAMPLER PROGRAMMING

Purpose:

This section outlines the initial programming setup for flow sampling from the flow-weighted composite (FWC) and discrete time interval (DTI) sampling units.

Equipment Overview:

At the time of preparing this document, there are three Auto-Samplers located in the basement of the Cape Cod Maritime Museum, one for the inlet to collect FWC samples, one for the outlet to collect FWC samples, and an additional one for the inlet to collect DTI samples, however may be relocated to the Chatham site at a later date.

Initial Programming:

As noted in the QAPP, intervals may be adjusted based on results obtained during the ongoing sampling program. As a starting point per the information provided in the Quality Assurance Project Plan (QAPP) prepared by EPA, samplers are pre-programmed to collect samples at the following intervals and quantities:

BMP Inlet (FWC)

Sample #	Time	Qty.	Sample #	Time	Qty.
1	5 min.	800 mL	13	9 hrs.	800 mL
2	15 min.	800 mL	14	10 hrs.	800 mL
3	30 min.	800 mL	15	11 hrs.	800 mL
4	60 min.	800 mL	16	12 hrs.	800 mL
5	2.0 hrs.	800 mL	17	13 hrs.	800 mL
6	2.5 hrs.	800 mL	18	14 hrs.	800 mL
7	3 hrs.	800 mL	19	15 hrs.	800 mL
8	4 hrs.	800 mL	20	16 hrs.	800 mL
9	5 hrs.	800 mL	21	18 hrs.	800 mL
10	6 hrs.	800 mL	22	20 hrs.	800 mL
11	7 hrs.	800 mL	23	22 hrs.	800 mL
12	8 hrs.	800 mL	24	24 hrs.	800 mL



BMP Outlet (FWC)

Sample #	Time	Qty.	Sample #	Time	Qty.
1	5 min.	800 mL	13	10 hrs.	800 mL
2	15 min.	800 mL	14	11 hrs.	800 mL
3	30 min.	800 mL	15	12 hrs.	800 mL
4	60 min.	800 mL	16	14 hrs.	800 mL
5	2 hrs.	800 mL	17	16 hrs.	800 mL
6	3 hrs.	800 mL	18	18 hrs.	800 mL
7	4 hrs.	800 mL	19	20 hrs.	800 mL
8	5 hrs.	800 mL	20	22 hrs.	800 mL
9	6 hrs.	800 mL	21	24 hrs.	800 mL
10	7 hrs.	800 mL	22	28 hrs.	800 mL
11	8 hrs.	800 mL	23	32 hrs.	800 mL
12	9 hrs.	800 mL	24	36 hrs.	800 mL

BMP Inlet (DTI)

Sample #	Container #	Time	Qty.
1	1	5 min.	800 mL
	2		800 mL
2	3	15 min.	800 mL
	4		800 mL
3	5	30 min.	800 mL
	6		800 mL
4	7	60 min.	800 mL
	8		800 mL
5	9	2 hrs.	800 mL
	10		800 mL



3. PRE-SAMPLING EVENT PROCEDURES

Purpose:

Follow the procedures below when visiting the site before an expected storm-sampling event to ensure all equipment is in operational order.

Step 1: Continually check the future weather forecast (i.e. National Weather Services, Weather Underground, etc.) for potential upcoming rain events. Recommended storm events will have durations of greater than six hours and less than 24 hours, and produce at least 0.35-inches of precipitation, thus triggering approximately 0.3-inches of stormwater runoff.

Step 2: Go to the Cape Cod Maritime Museum located at 135 South Street, Hyannis, MA or near the intersection of South Street and Pleasant Street in Barnstable, MA. Sampling equipment is located in the basement, nearest the external door behind the boat shed. See photos below.



Figure 1: Auto-Samplers



Figure 2: Signature Flow Meters

Step 3: Unfasten the 3 metal bottom latches on the Auto-Sampler, remove the top from the base and set aside to expose the carousel with sample bottles and holders. Caution! Top may be heavy.

Step 4: Ensure that the base is filled with sample bottles and holders are properly locked in place. The base should contain 24 1-liter sample bottles. Replace any missing bottles if necessary and ensure that no caps are in place.

Step 5: Gently test the distributor arm at the bottom of the base cover to ensure proper alignment.

Step 6: Carefully replace the top section of the Auto-Sampler onto the base and re-latch the cover in place. Caution! Top may be heavy.

Step 7: Open the rubber top latches on the Auto-Sampler to access the control panel, remove the cover and set aside.

- The Auto-Sampler should be connected to the Signature Flow Meter and plugged into an outlet on the wall.
- Press the Home button and observe the display screen on the Auto-Sampler to ensure that it is powered on.

Step 8: Turn on the Auto-Sampler by pressing the ⓘ button.

Step 9: Carefully replace the Auto-Sampler cover and re-latch the cover in place.

Step 10: Verify that the Signature Flow Meter is on.

- The Signature Flow Meter should be plugged into an outlet on the wall.
- Press the Home button and observe the display screen on the Signature Flow Meter to ensure that it is powered on.

Step 11: Repeat the above procedures for the remaining Auto-Samplers and Signature Flow Meter.

Step 12: Notify Ray Cody, EPA about the potential rainfall event and anticipated stormwater samples (see included Contact Sheet).



4. SAMPLING PROCEDURES

Purpose:

Follow the procedures below when retrieving samples after a storm event. Due to the extended duration of the sampling program, these tasks should take place at least 24-hours after the storm-sampling event is completed, but not longer than 48-hours.

Step 1: Go to the Cape Cod Maritime Museum located at 135 South Street, Hyannis, MA or near the intersection of South Street and Pleasant Street in Barnstable, MA. Sampling equipment is located in the basement, nearest the external door behind the boat shed. See photos below.



Figure 1: Auto-Samplers



Figure 2: Signature Flow Meters

Step 2: Inspect the general condition of all of the equipment, tubing, casing, cables, and sensors. Note any irregularities.

Step 3: Open the rubber top latches on the Auto-Sampler to access the control panel, remove the cover and set aside.

Step 4: Turn off the Auto-Sampler by pressing the  button.

Step 5: Unfasten the 3 metal bottom latches on the Auto-Sampler, remove the top from the base and set aside to expose the carousel with sample bottles and holders. Caution! Top may be heavy.

Step 6: Carefully screw covers onto each sample bottle to prevent sample spillage. Note that sample bottles will be aligned with a number on the side of the carousel.

- The inlet FWC Auto-Sampler should be filled with 24 sample bottles, however may not fill all bottles depending on storm duration and flow characteristics.
- The outlet FWC Auto-Sampler should be filled with 24 sample bottles and should always fill all 24 bottles with samples. If not, then it is possible that the Auto-Sampler has not yet finished sampling the entire storm.
- The DTI Auto-Sampler should always use 10 sample bottles, consisting of 5 samples (1 sample per 2 bottles).

Step 7: Remove the full carousel for transport back to the office for Post-Sampling and Sample Preparation Procedures under a separate SOP. Be sure to keep track of which carousel came from which sampler! Carousels and samplers are labeled.

Step 8: Replace the full carousel with an empty carousel filled sample bottles and holders per the Post-Sampling and Sample Preparation Procedures SOP. Ensure that no caps are present.

Step 9: Carefully replace the top section of the Auto-Sampler onto the base and re-latch the cover in place. Caution! Top may be heavy.

Step 10: Compare bottles within the full carousel and ensure the correct bottles were filled according to the Auto-Sampler readings. Note any irregularities.

Step 11: Visually check to see if the sample volume appears to correspond with sample bottles according to the Auto-Sampler readings. Note any irregularities.

Step 12: Carefully replace the Auto-Sampler cover and re-latch the cover in place.

Step 13: Download all data from the Signature Flow Meter and save the data on the provided USB thumb drive for this monitoring program.



- Connect a USB thumb drive to the micro-USB cable, available within the Signature Flow Meter cover. A *USB Options* menu will automatically appear.
- If not, select the Menu option on the Home screen. (Note: *USB Options* menu will only appear if a USB connection is detected by the Signature Flow Meter).
- Select USB Options > Retrieve Text Reports or Retrieve Data.
- Retrieve Text Reports allows you to download all reports, all reports since a certain date, or all reports in a time/date range.
- Retrieve Data allows you to download all data, all data since a certain date, or data in a time/date range.
- Be sure to check the box to export or save as a .csv file (comma separated value), as this can be read in Excel.
- To download Text Reports or Data, press Enter on the desired option and leave the USB thumb drive connected to the Signature Flow Meter during the downloading process. This will download data such as flow in and out of the BMP, data collected automatically via the sondes, and the time and date of each measurement.
- Make sure to leave USB thumb drive connected until it is fully complete. It can stay at 100% for some time (10 minutes at times) to complete data download and conversion.
- Take the USB thumb drive to a computer and email the file(s) to: Ray Cody, EPA, cody.ray@epa.gov. Data will in part be used to calculate FWC aliquots.

Step 14: Document the following after each storm sampling event in a field book or field data sheets:

- Take note of the total number of samples collected at each sampling site including the FWC grab samples, DTI grab samples, and any other in-situ sampling.
- Record the number of samples that were unsuccessfully collected.
- Record the time of the first sample and the last sample and take note of when the rainfall and stormwater runoff ended.
- Note any excess sedimentation or debris accumulation on and around the equipment and sampling location.
- Note any maintenance activities that need to be followed-up before the next storm-sampling event.

Step 15: Proceed to Post-Sampling and Sample Preparation Procedures SOP.



5. POST-SAMPLING AND SAMPLE PREPARATION PROCEDURES

Purpose:

Follow the procedures below after a sampling event has been completed in order to prepare samples to send to the EPA laboratory for analysis.

Step 1: Bring the full carousels to an appropriate work area with plenty of space and light. Take care not to mix up carousels or alter the location of sample bottles within the carousel.

For samples to be flow-weighted composited, proceed to Step 2. For discrete samples, proceed to Step 3.

Step 2: This step should only be performed for samples to be flow-weighted composited. Discrete samples should not be composited! Flow-weighted composite (FWC) grab samples will need to be composited by the Town in-house before shipping to EPA.

Once flow data has been sent to EPA as outlined under the Sampling Procedures SOP, EPA will respond with instructions on how to flow-weight the composite samples. This will be performed by collecting a certain volume aliquot from each sample bottle and compositing them into a single EPA-provided bottle to represent the entire storm event.

Prior to uncapping and collecting any sample aliquot, gently shake the sample bottle to ensure water within is thoroughly mixed. Thoroughly rinse and sanitize any instruments between aliquots.

Note that two bottles will be needed, 1 for TN/TP and 1 for TSS, so each sample will have 2 aliquots of equal volume collected, one for each EPA-provided sample bottle.

Label each one as follows:

<site> <sample type> <analyte> <location> <date; time>

- site: B = Barnstable



- sample type: FWC = Flow-weighted composite samples
- analyte:
 - TN/TP = Total Nitrogen / Total Phosphorus
 - TSS = Total Suspended Solids
- location:
 - I = Inlet
 - O = Outlet
- date; time:
 - mm/dd/yy; hour:min (where time is time at which the storm began)

Example: A TN/TP sample collected from the outlet FWC sampler at the Barnstable BMP outlet at 7:57 PM on October 17, 2017 after storm initiation recorded as 6:10 PM: “B FWC TN/TP O 10-17-17; 6:10 PM”.

Proceed to Step 4.

Step 3: This step should only be performed for discrete samples. Note that the Signature Flow Meter records the time, data, and bottle number, indexed off of the inside of the Auto-sampler. Carefully remove each sample bottle from the base of the Auto-Sampler and pour into a 1-liter EPA-provided bottle, taking careful note of the indexed number within the carousel. Label each one as follows:

<site> <sample type> <analyte> <location> <date; time> <sample #> <time>

- site: B = Barnstable
- sample type: DTI = Discrete-time interval grab sample
- analyte:
 - TN/TP = Total Nitrogen / Total Phosphorus
 - TSS = Total Suspended Solids
- location:
 - I = Inlet
 - O = Outlet
- date; time:
 - mm/dd/yy; hour:min (where time is time at which the storm began)
- sample #:



- $n = 1, 2, 3 \dots n$ (where n is the bottle number or sequential order of the sample from when the storm initiated)
- time:
 - t = time after the storm initiated

Example: A TSS sample collected from the inlet DTI sampler on the floor at the Barnstable BMP inlet 1:06 PM on November 21, 2017 after storm initiation recorded as 12:15 PM: "B DTI TSS I 11-21-17 12:15 PM n 1:06 PM", where n is the bottle number.

Proceed to Step 4.

Step 4: Once all samples have been transferred into properly-labeled EPA-provided bottles, they are ready for shipping to EPA. Complete a Chain of Custody:

- Transfer sample labels and times per Steps 3 and 4 onto the Chain of Custody.
- Fill out the Chain of Custody with date, contact information, signature, etc.
- Place the completed Chain of Custody in a one-gallon plastic bag to keep it dry and tape it to the inside lid of the sample cooler.

Carefully place all labeled EPA-provided sample bottles in a cooler with ice packs, resealable plastic bags filled with ice, or plastic bags sealed with a twist-tie filled with ice.

Step 5: Ship or deliver the samples to EPA's National Exposure Research Laboratory (NERL) for analysis with the finalized Chain of Custody.

- Notify Ray Cody, EPA about the potential rainfall event as early as possible (see included Contact Sheet)
- If the samples are being shipped, FedEx the samples overnight using EPA's FedEx account number.
- The samples may be shipped Monday through Thursday. Note that the NERL is closed on Saturday and Sunday, and thus samples collected on a Friday should be mailed out on Monday.

The mailing address is:

EPA New England Regional Laboratory



11 Technology Drive
North Chelmsford, MA, 01863-2431

- If the samples are being delivered, they may be delivered Monday-Friday. The lab is located at 11 Technology Drive, North Chelmsford, MA.

Step 6: Clean and decontaminate all sample bottles for replacement within the carousel.

- Clean with tap water and phosphate-free laboratory detergent, such as 2% Liquinox® or Alconox® and tap water solution.
- Rinse thoroughly with tap water to remove all soap solution.
- Rinse a minimum of three times with analyte free/DI water.
- Allow to air dry completely.

Step 7: Refill sampling carousel for deployment during the next sampling round.

- Once bottles are dry, reinsert all bottles into the carousel and ensure that all bottles and holders are properly locked in place.
- Cover bottles with screw-on caps to prevent atmospheric deposition of contaminants. Each base should contain 24 1-liter sample bottles.
- Store safely for redeployment after the next sampling round.



6. ANNUAL PROGRAM STARTUP GUIDELINES

Purpose:

Conduct the following protocols at least once per year, likely during the initial spring sampling program startup. Upon completion, routine bi-annual maintenance should be performed as outlined in Section 7.

Program Data: Before the first sampling round, download the Current Program settings on the provided USB thumb drive.

- Connect a USB thumb drive to the micro-USB cable, available within the Signature Flow Meter cover. A *USB Options* menu will automatically appear.
- Select the Menu option on the Home screen. (Note: *USB Options* menu will only appear if a USB connection is detected by the Signature Flow Meter).
- Select USB Options > Save Current Program.
- To download the current programming settings, press Enter and leave the USB thumb drive connected to the Signature Flow Meter during the downloading process.

Equipment Connections: Check that the Rain Gauge, Bubbler/Calibrated Flume, and Area-Velocity Sensor are properly connected and functioning using the Signature Flow Meter.

- On the home screen, select the Menu option.
- Select Hardware Setup > Smart Sensor Setup (TIENet)
- This option will display the most recently detected TIENet devices connected to the Signature Flow Meter. If a device is missing, highlight Perform Scan and press Enter.
- Missing Sensors will indicate any previously connected devices that are no longer detected. Select Retain to keep identification information.
- Replaced Sensors displays any newly added sensors that have replaced a Missing Sensor which information had been retained.
- Additional Sensors displays newly added devices.

Sonde Deployment: If sondes were previously removed for calibration, they should be reinstalled and properly connected prior to commencing sampling events.



7. ROUTINE BI-ANNUAL MAINTENANCE GUIDELINES

Purpose:

Conduct the following maintenance protocols on each piece of equipment as specified below or as needed. See attached [Figure 3](#) at the end of this section for the location of all equipment at the Barnstable BMP.

Note that this section outlines routine maintenance only, expected to be encountered during regular operations. For detailed maintenance, or explanations on how to correct an error message, please refer to individual product documentation.

Signature Flow Meter: Conduct maintenance at least twice per year.

- The Signature Flow Meters located inside the Cape Cod Maritime Museum. Access should be through the rear entrance.
- Check condition of desiccant pack, located on the left side of the Signature Flow Meter.
 - Spent desiccant is dark in color whereas fresh desiccant is orange.
 - If desiccant is mostly dark, slide the plastic container off the unit and replace with fresh orange desiccant.
- Open Signature Flow Meter cover to access the screen, keyboard, and USB connections.
- Ensure that the Signature Flow Meter is clear of snow, ice, debris, and sediment.
- Check all cables, tubes, and electrical connections for tightness and/or damage. Replace or conduct maintenance as needed.
- Check the tubing for the Bubbler/Calibrated Flume for kinks/twists and ensure that the tubing is clean of sediment and debris. Straighten, clean, or replace tubing as needed.



- Ensure that the Signature Flow Meter is properly connected to the Bubbler/Calibrated Flume, Area-Velocity (AV) Sensor, Auto-Sampler, and Rain Gauge.
 - On the home screen, select the Menu option.
 - Select Hardware Setup > Smart Sensor Setup (TIENet)
 - This option will display the most recently detected TIENet devices connected to the Signature Flow Meter. If a device is missing, highlight Perform Scan and press Enter.
 - Missing Sensors will indicate any previously connected devices that are no longer detected. Select Retain to keep identification information.
 - Replaced Sensors displays any newly added sensors that have replaced a Missing Sensor which information had been retained.
 - Additional Sensors displays newly added devices.
- Check for the presence of error messages or other abnormal conditions.

ISCO 6712 Sampler: Conduct maintenance at least twice per year.

- The Auto-Samplers are located inside the Cape Cod Maritime Museum. Access should be through the rear entrance.
- The three samplers are located on the floor and are labeled as either FWC inlet, FWC outlet, or DTI inlet.
- Ensure that the ISCO 6712 Sampler, or Auto-Sampler, is clear of snow, ice, debris, and sediment.
- Check suction tubing for kinks/twists and ensure that the tubing is clear of sediment and debris. Straighten, clean, or replace tubing as needed.
- If suction tubing/pump tubing needs to be cleaned using a weak acid such as hydrochloric acid (HCl) and then rinsed with deionized (DI) water.
- Check the humidity indicator by viewing the Internal Case humidity located to the right of the keypad. The paper indicator is blue when the control box is dry. If moisture does accumulate, the numbered areas will turn light pink



or white. If the 30% area of the humidity indicator turns light pink or white, open the control unit, inspect it for leaks, and replace the desiccant bag (part 099-0002-00).

- Check the condition of the peristaltic pump. Replace or conduct maintenance as needed.
- Check that all tubing is properly clamped and connected to the Auto-Sampler.
- Check all electrical connections for tightness and ensure that there is no corrosion and/or damage. Replace as needed.
- Check the strainer and ensure that it is clear of sediment and debris. Clean or replace as needed.
- Ensure that the Auto-Sampler is connected to the Signature Flow Meter.
- Check for the presence of error messages or other abnormal conditions.

Rain Gauge: Conduct maintenance at least twice per year.

- The Rain Gauges are located on top of the Cape Cod Maritime Museum roof, near the rear entrance. A ladder will be required for access.
- Ensure that the exterior of the Rain Gauge is clear of snow, ice, debris, sediment, and bird droppings.
- Loosen the two metal thumb screws and remove the top. Ensure that the interior is free of debris buildup.
- Make sure the Rain Gauge “tipper” can move freely. Note that by moving the “tipper”, the Rain Gauge will think it’s raining. The “tipper” should only be touched when both the Auto-Sampler and Signature Flow Meter are unplugged.



Calibrated Flume/Bubbler: Conduct maintenance at least twice per year.

- The Calibrated Flumes are located at the inlet and outlet pipes of the BMP. They can be accessed by removing the drainage manhole covers.
- Ensure that the Calibrated Flume is clear of snow, ice, debris, and sediment.

- Check that the Bubbler tubing is connected to the Signature Flow Meter.

Area-Velocity Sensor: Conduct maintenance at least twice per year.

- The Area-Velocity Sensors are located at the inlet and outlet pipes of the BMP. They can be accessed by removing the drainage manhole covers.
- Ensure that the AV Sensor is clear of snow, ice, debris, and sediment.
- Check that the AV Sensor is connected to the Signature Flow Meter.



Aqua-Troll 600 Multiparameter Sonde: Conduct maintenance at least twice per year.

- The Aqua TROLL sondes are located within the diversion structure on the inlet side and within the outlet control structure on the outlet side. They can be accessed by removing the drainage manhole covers.
- Note that sondes are equipped with a motorized sensor wiper that will automatically clean the probes and reduce the need for maintenance.
- Clean the sonde with warm water and soap, then rinse.
- Gently rinse and clean around probes with a soft cloth or swab. Do not use harsh solvents or soaps.
- The pH probe has a fill solution that must be replaced every 5 to 6 months, or if adverse operation is noted (see product manual for instructions).
- Ensure pH probe is kept submerged at all times, even if removed for storage.
- Check dessicant kit and replace when it changes from purple to pink (Maintenance Kit 0078940).
- Note that for best results, the sonde should be sent to the factory for calibration every 12 to 18 months.



8. CONTACT INFORMATION

Name, Organization	Project Role	Email Address	Phone #
Sampling Team Leader			
Dale Saad, Ph.D., Town of Barnstable	Senior Project Manager, Barnstable DPW	dale.Saad@town.barnstable.ma.us	508-790-6400 X4941
EPA Contacts			
Ray Cody, EPA	EPA Project Manager	cody.ray@epa.gov	617-918-1366
Mark Voorhees, EPA	EPA/OEP Technical Advisor	voorhees.mark@epa.gov	617-918-1537
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Comprehensive Environmental Inc.			
Nick Cristofori, PE, CEI	CEI Project Engineer	ncristofori@ceiengineers.com	603-261-3054
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Carl Cederberg	Equipment Specialist	carl.cederberg@teledyne.com	402-465-3025



9. TROUBLESHOOTING PROGRAMMING PROCEDURES

System error or failure occurred.

- Connect the provided USB thumb drive to the Signature Flow Meter with a micro-USB cable, available within the Signature Flow Meter cover.
- Select the Menu option on the Home screen.
- Select USB Options > Gather Fault Data (Note: The *USB Options* menu will only appear if a USB connection is detected by the Signature Flow Meter).
- This option is capture Flow Meter activity before and during a system error or failure.
- To download fault data, press Enter and leave the USB thumb drive connected to the Signature Flow Meter during the downloading process.
- Gathering fault data will help to troubleshoot why there was a system error or failure.

Signature Flow Meter accidentally reset to default factory settings.

- Connect the provided USB thumb drive to the Signature Flow Meter with a micro-USB cable, available within the Signature Flow Meter cover.
- Select the Menu option on the Home screen.
- Select USB Options > Load Existing Program (Note: The *USB Options* menu will only appear if a USB connection is detected by the Signature Flow Meter).
- This option overwrites the existing program settings with a saved program. Upload the original program settings that were saved during the first sampling event.
- To upload the program settings, press Enter and leave the USB thumb drive connected to the Signature Flow Meter during the uploading process.

To ***view*** or ***download diagnostic reports***.

- Connect the provided USB thumb drive to the Signature Flow Meter with a micro-USB cable, available within the Signature Flow Meter cover.
- Select the Menu option on the Home screen.
- Select Administration > Sensor Diagnostics (Note: To download diagnostic reports, a USB connection must be detected by the Signature Flow Meter).
- This option allows you to view or download system diagnostic reports.



- To download the diagnostic reports, press Enter and leave the USB thumb drive connected to the Signature Flow Meter during the downloading process.

To **view** or **download Signature Flow Meter information**.

- Select the Menu option on the Home screen.
- To view Signature information, select Administration > Display Signature Information
- This option displays the serial number, model number, software version, and hardware version of the Flow Meter and all connected devices.
- To download Signature information, connect the provided USB thumb drive to the Signature Flow Meter with a micro-USB cable, available within the Signature Flow Meter cover.
- Select Home > Menu > USB Options > Save Signature Information.
- Press Enter and leave the USB thumb drive connected to the Signature Flow Meter during the downloading process.



Green Infrastructure Stormwater Nitrogen BMP

Attachment A

BARNSTABLE, MA BACTERIA SAMPLING SOPs

Intersection of South Street and Pleasant Street

Barnstable, MA

Contents

1. Colilert® Presence/Absence Test Standard Operating Procedure (SOP)..... 2
2. Enterolert® Standard Operating Procedure (SOP)..... 5
3. Membrane Filtration Standard Operating Procedure (SOP) 8



1. Colilert® Presence/Absence Test Standard Operating Procedure (SOP)

Reference: Standard Methods SM9223 (20th Edition).

IDEXX Laboratories Colilert® product insert.

IDEXX Laboratories Colilert® Presence/Absence Comparator product insert.

A. Purpose

Colilert® simultaneously detects total coliform and *E. coli* in water. When total coliforms metabolize Colilert's nutrient-indicator, ONPG, the sample turns yellow. When *E. coli* metabolizes Colilert's nutrient-indicator, MUG, the sample fluoresces.

B. Storage of Media

Store at 2-30°C away from light in original packaging. Refer to package for expiration date.

C. Test Bottle Sterility Check

At least one bottle per lot of commercially obtained, pre-sterilized Colilert bottles (WV120SB-200) shall be checked for sterility by adding approximately 25 ml of sterile non-selective broth media to each bottle. The bottle shall be capped and rotated so that the broth comes in contact with all surfaces and shall be incubated at 35°C±0.5°C for 24 hours prior to checking for growth. Sterility check results shall be recorded in a bound book. Bottles from each lot shall not be used unless satisfactory results are obtained from each tested bottle. In addition, all manufacturer certificates of sterility shall be retained on file for a minimum of 5 years.

D. Procedure

1. Disinfect work area with 70% ethanol solution.
2. Label Colilert test bottle (WV120SB-200) with sample laboratory ID number.
3. Shake sample vigorously for 15 sec (approximately 25 times) to mix.
4. Aseptically add 100 ml of sample to the Colilert® test bottle using the fill-to-line on the bottle.
5. Add contents of one pack of the Colilert® reagent for 100 ml sample (WP020) to the Colilert® test bottle containing the water sample and cap the bottle.
6. Shake and invert the bottle to dissolve, the solution will appear clear.
7. Incubate the bottle at 35° C ±0.5° C for 24 hours.
8. Record the date/time/analyst that sample analysis is completed on the Potable Water Test Worksheet.
9. Read results according to Table B1 below.



- a. *Total coliform bacteria*: Examine bottle(s) for chromogen (color) reaction according to Table B1 below. If a chromogenic response is questionable at 24 hrs, incubate up to an additional 4 hr (total time of incubation shall not exceed 28 hrs). If the chromogen intensifies, the sample is total coliform positive, if it does not, the sample is negative.
- b. *Escherichia coli*: Examine positive total coliform bottle(s) (yellow) for fluorescence using a long-wavelength ultraviolet light (6-watt, 366 nm) directed at the sample from a distance of 5-6 inches in a darkened area. Compare against the reference comparator. The presence of fluorescence is a positive test for *E. coli*. If fluorescence is questionable, incubate for an additional 4 hr, intensified fluorescence is a positive test result.

Table B1

Appearance	Interpretation
No color	Negative for Total Coliform and <i>E. coli</i>
Less yellow than the comparator (WP104)	Incubate up to an additional 4 hours and re-check
Yellow equal to or greater than the comparator	Positive for Total Coliforms
Yellow, and fluorescence equal to or greater than the comparator	Positive for <i>E. coli</i>

10. Record result as presence or absence on Potable Water Test Worksheet.
11. Record date/time/analyst that results were read on the Potable Water Test Worksheet.
12. The Laboratory Director will promptly notify client of a positive Total Coliform/*E. coli* test and request re-sample.
13. Discard all test materials by placing in an autoclave-biohazard bag. Seal bag with autoclave indicator tape and autoclave for 30 min at 121° C. Allow to cool, place in garbage bag and discard in regular trash.

E. Interpretation

Colilert® results are definitive at 24-28 hours. In addition, positives for both total coliforms and *E. coli* observed before 24 hours and negatives observed after 28 hours are also valid.

F. Quality Control

Each new lot of Colilert® media shall be tested for proper performance.

1. Label 4 Colilert® test bottles, one each for *E. coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and a blank.
2. Add 100 ml sterile reagent water to each of the Colilert bottles.



3. Inoculate appropriate container with loopful of stock culture.
4. Follow section D., steps 5 to 9 above.
5. Results should be as follows:

E. coli - yellow with positive fluorescence.

Klebsiella - yellow with negative fluorescence.

Pseudomonas - clear with negative fluorescence.

Blank – clear with negative fluorescence.



2. Enterolert® Standard Operating Procedure (SOP)

Reference: IDEXX Laboratories Enterolert® product insert.

IDEXX Laboratories Quanti-Tray/Quanti-Tray 2000 product inserts.

IDEXX Laboratories Quanti-Tray Sealer Model 2X User Manual

A. Purpose

Enterolert™ detects enterococci such as *E. faecium* and *E. faecalis* in fresh and marine water. When enterococci utilize their B-glucosidase enzyme to metabolize Enterolert's nutrient-indicator, 4-methyl-umbelliferyl B-D-glucoside, the sample fluoresces. Enterolert detects enterococci at 1 cfu per 100 ml sample within 24 hours.

B. Storage of Media

Store at 2-30°C away from light in original packaging. Refer to package for expiration date.

C. Test Bottle Sterility Check

At least one bottle per lot of commercially obtained, pre-sterilized IDEXX bottles (WV120SB-200) shall be checked for sterility by adding approximately 25 ml of sterile non-selective broth media to each bottle. The bottle shall be capped and rotated so that the broth comes in contact with all surfaces and shall be incubated at 35°C ± 0.5°C for 24 hours prior to checking for growth. Sterility check results shall be recorded in a bound book. Bottles from each lot shall not be used unless satisfactory results are obtained from each tested bottle. All manufacturer certificates of sterility shall be retained on file for a minimum of 5 years.

D. Procedure

D.1 Presence/Absence (P/A) Procedure

1. Disinfect work area with 70% ethanol solution.
2. Label IDEXX test bottle (WV120SB-200) with sample laboratory ID number.
3. Shake sample vigorously for 15 sec (approximately 25 times) to mix.
4. Aseptically add 100 ml of sample to the test bottle using the fill-to-line on the test bottle.
5. Add contents of one pack of the Enterolert reagent for 100 ml sample to the test bottle containing the water sample and cap the bottle.
6. Shake and invert the bottle to dissolve.
7. Incubate the bottle at 41°C ± 0.5°C for 24 hours.
8. Record the date/time/analyst that sample analysis is completed and the Enterolert Lot No. and Expiration Date on the field sheet.
9. Read results according to Table B2 below.



10. Record result as presence or absence on Enterolert Worksheet.
11. Record date/time/analyst that results were read on the Enterolert Worksheet.
12. Discard all test materials by placing in an autoclave-biohazard bag. Seal bag with autoclave indicator tape and autoclave for 30 min at 121° C. Complete relevant entries in autoclave log. Allow to cool, place in garbage bag and discard in regular trash.

D.2 Quanti-Tray/Quanti-Tray 2000® Enumeration Procedure

1. Disinfect work area with 70% ethanol solution.
2. Label IDEXX test bottle (WV120SB-200) with sample laboratory ID number.
3. Shake sample vigorously for 15 sec (approximately 25 times) to mix.
4. Aseptically add 100 ml of sample to the test bottle using the fill-to-line on the bottle.
5. Add contents of one pack of the Enterolert reagent for 100 ml sample to the test bottle containing the water sample and cap the bottle.
6. Shake and invert the bottle to dissolve.
7. Pour sample/reagent mixture into a Quanti-Tray or Quanti-Tray 2000 and seal in IDEXX Quanti-Tray Sealer.
 - a. Use one hand to hold a Quanti-Tray (Quanti-Tray 2000) upright with the well side facing the palm.
 - b. Squeeze the upper part of the Quanti-Tray so that the Quanti-Tray bends toward the palm.
 - c. Open the Quanti-Tray by pulling the foil tab away from the well side. Avoid touching the inside of the foil or tray.
 - d. Pour the reagent/sample mixture directly into the Quanti-Tray, avoiding contact with the foil tab. Allow foam to settle.
 - e. Place the sample-filled Quanti-Tray onto the rubber tray carrier of the Quanti-Tray Sealer with the well side (plastic) of the Quanti-Tray facing down to fit into the carrier.
 - f. Seal according to the Quanti-Tray Sealer instructions.
8. Place the sealed tray in a 41° C \pm 0.5° C incubator for 24 hours.
9. Record the date/time/analyst that sample analysis is completed and the Enterolert Lot No. and Expiration Date on the field sheet.
10. Read the results according to Table B2. Count the number of positive wells and refer to the Quanti-Tray or Quanti-Tray 2000 MPN table to obtain the Most Probable Number (MPN).
11. Record number of positive wells and MPN on the Enterolert worksheet.
12. Record date/time/analyst that results were read on the Enterolert Worksheet.
13. Discard all test materials by placing in an autoclave-biohazard bag. Seal bag with autoclave indicator tape and autoclave for 30 min at 121° C.



Complete relevant entries in autoclave log. Allow to cool, place in garbage bag and discard in regular trash.

E. Interpretation

Table B2

Appearance	Interpretation
Lack of fluorescence	Negative for enterococci
Blue fluorescence	Positive for enterococci

1. Look for fluorescence with a 6-watt, 365 nm, UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and towards the sample
2. Enterolert results are definitive at 24-28 hours. In addition, positives for enterococci observed before 24 hours and negatives observed after 28 hours are also valid.

F. Procedural Notes

1. Marine water samples must be diluted at least tenfold with sterile fresh water. Multiply the MPN value by the dilution factor to obtain the proper quantitative result.
2. Use only sterile, nonbuffered, oxidant-free water for dilutions.
3. For comparison, a water blank can be used when interpreting results.
4. Enterolert is a primary water test. Enterolert performance characteristics do not apply to samples altered by any pre-enrichment or concentration.

G. Quality Control

Each new lot of Enterolert media shall be tested for proper performance.

1. Label 4 test bottles, one each for *Enterococcus faecium*, *Serratia marcescens*, *Aerococcus viridans*, and a blank.
2. Add 100 ml sterile water to each of the test bottles.
3. Inoculate appropriate test bottle with loopful of stock culture.
4. Follow section D.1, steps 5 to 12 above.
5. Results should be as follows:

Enterococcus faecium (ATCC #35667) - fluorescence.

Serratia marcescens (ATCC #43862) - no fluorescence.

Aerococcus viridans (ATCC #10400) - no fluorescence.

Blank - clear with no fluorescence.



3. Membrane Filtration Standard Operating Procedure (SOP)

Reference: Standard Methods SM9222B (20th Edition)

Improved Enumeration Methods for the Recreational Water Quality Indicators:
Enterococci and Eschericia coli (US EPA/821/R-97/004)

HACH Membrane Filtration: Coliforms, Enterococci and Pseudomonas

A. Purpose

The Membrane filtration (MF) method is a fast, simple way to estimate bacterial populations in water that is low in turbidity. The type of bacteria enumerated can vary depending on the medium used and the incubation conditions.

B. Storage of Media

Store at 2-8° C away from light in original packaging. Refer to package for expiration date. See individual medium for any specific storage conditions.

C. Dilutions

- Select dilution(s) such that the total number of colonies on a filter will be between 20 and 80.
- The sample volume for potable water samples shall be 100 ml.
- The sample volume for marine bathing beaches shall be 30 ml and for freshwater beaches 100 ml. These volumes may be altered depending on sample turbidity.
- Guidance for sample volumes for other sample sources is provided in Table SM9222:I.
- All samples, except potable water, are run in duplicate for each sample volume to be filtered. Potable water samples are run as a single 100 ml plate.
- For all volumes less than 100 ml use the smallest pipette for the volume being delivered. For sample volumes of 10 ml or less add approximately 10 ml of sterile dilution water to the filter before adding sample. For sample volumes of less than 1 ml make up appropriate dilutions using sterile, 99 ml dilution water blanks.

D. Procedure

1. Disinfect work area with 70% ethanol solution.
2. Check that filter trap is empty; if not, empty into sink.
3. Use sterile filtration units at the beginning of each filtration series as a minimum precaution to avoid accidental contamination. A filtration series is considered to be interrupted when an interval of 30 min or longer elapses between sample filtration. After such interruption, treat any further sample filtrations as a new filtration series and sterilize all membrane filter holders in use.
 - a. For potable water samples sterilize filter funnels and bases (separately) by sealing in Instant Sealing Sterilization Pouch (Fisherbrand, 01-812-55) and autoclaving for 15 min at 121° C.



- b. For other than potable water samples, the alternative method of sterilization using UV light may be used. At the start of each filtration series sterilize filter funnels and bases in UV light box for at least 2 minutes. CAUTION UV light is carcinogenic and damaging to eyes and skin.
4. Label underside (media side) of each plate with sample laboratory ID number and volume of sample or dilution.
5. Remove filter unit bases from sterilization pouch or UV box and place in manifold, exercising care not to touch top surface of filter base.
6. Using sterile (dip in 95% ethanol and flame off), flat-tipped forceps remove filter (Gelman GN-6, 47 mm, 0.45 um) from wrapper and center filter, grid side up, on filter base. Touch only outer edge of filter with forceps.
7. Remove filter funnel from sterilization pouch or UV box touching only outer surface and attach to base (magnetic) taking care not to dislodge filter.
8. A start and finish membrane filtration control test of rinse water (100ml), medium, and supplies shall be conducted for each filtration series. If sterile controls indicate contamination, all data on samples affected must be rejected and immediate re-sampling requested.
9. Shake sample vigorously for 15 sec (approximately 25 times) to mix.
10. Aseptically place the appropriate amount of sample into funnel.
 - a. For potable water samples the sample volume shall be 100 ml. The 100 ml fill line on the filter apparatus will be used to measure this volume having been previously checked against a graduated cylinder.
 - b. For other waters the sample volume will vary depending on the expected bacterial density. Sample volumes of less than 100 ml will be measured using the smallest volume appropriate sterile, disposable pipet. An ideal sample volume will yield 20 to 80 target colonies. When less than 10 ml of sample is to be filtered, add approximately 10 ml of sterile dilution water to the funnel before adding the sample. This increase in water volume aids in uniform dispersion of the bacterial suspension over the entire effective filtering surface.
11. Turn on vacuum pump using wall switch. Open valve on manifold and filter sample under vacuum (15 psi or less). After sample has filtered through, aseptically rinse interior surface of funnel with sterile dilution water (10-15 ml) from squeeze bottle.
12. Close valve on manifold, remove funnel and with sterile forceps remove filter from filter base, being careful to touch only the outer edge of filter. Using a rolling motion to avoid entrapment of air place the filter on the appropriate medium. If air spaces between the medium and filter are noted, pick up edge of filter and re-seat to remove air pockets.
13. Invert the plates (i.e. media on top), place in stacks of two and incubate at the appropriate temperature according to Table B3 below.



14. Record the date/time/method/analyst that sample analysis was completed on the Membrane Filtration Worksheet.
15. Read results according to Table B3 below. Count plates with the aid of a low-power (10 – 15 x magnification) binocular wide-field dissecting microscope with a cool white fluorescent light source directed to provide optimal viewing of sheen/colonies. (see Standard Methods 9222 B for additional guidance)
 - a. *Potable Water:* If confluent growth occurs, covering either the entire filtration area of the membrane or a portion thereof, and colonies are not discrete, report results as “confluent growth with (or without) coliforms.” If the total number of bacterial colonies, coliforms plus noncoliforms, exceeds 200 per membrane, or if the colonies are not distinct enough for accurate counting, report results as “too numerous to Count” (TNTC) or “confluent,” respectively.

For potable water, the presence of coliforms in such cultures showing no sheen may be confirmed by either transferring a few colonies or placing the entire membrane filter culture into a sterile tube of brilliant green lactose bile broth. As an alternative, brush the entire filter surface with a sterile loop, applicator stick or cotton swab and inoculate this growth to the tube of brilliant green lactose bile broth. If gas is produced from the brilliant green bile broth tube within 48 hr at $35 \pm 0.5^{\circ}\text{C}$, coliforms are present. For compliance with the EPA Total Colifom Rule, report confluent growth or TNTC with at least one detectable coliform colony (which is verified) as a total coliform positive sample.

Report confluent growth or TNTC without detectable coliforms as invalid. For invalid samples, request a new sample from the same location within 24 hrs and select more appropriate volumes to be filtered per membrane, observing the requirement that the standard drinking water portion is 100 ml, or choose another coliform method that is less subject to heterotrophic bacterial interferences.

- b. *Non-potable water:* Report confluent growth or membranes with colonies too numerous to count as described in *a* above. Request a new sample and select more appropriate volumes for filtration.



Table B3

Test	MF Medium	Incubation	Typical Appearance
Total Coliform (potable water only)	mEndo Agar LES	35° C \pm 0.5° C; 22-24 hrs	Pink to dark red with metallic surface sheen
Enterococci	m-EI	41° C \pm 0.5° C; 24 hrs	Any color with a blue halo
<i>E. coli</i>	m-TEC	35° C \pm 0.5° C; 2 hrs 44.5° C \pm 0.2° C; 22 hrs	Yellow or yellow brown after Urea Substrate test*
<i>E. coli</i>	Modified m-TEC	35° C \pm 0.5° C; 2 hrs 44.5° C \pm 0.2° C; 22 hrs	Red to magenta

* Prepare Urea Substrate Medium and perform test as per Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and Eschericia coli (US EPA/821/R-97/004)

16. Record results on Membrane Filtration Worksheet.
17. Record date/time/analyst that results were read on the Membrane Filtration Worksheet.
18. The Laboratory Director will promptly notify client of a positive Total Coliform test(s) from potable water samples.
19. Discard all test materials by placing in an autoclave-biohazard bag. Seal bag with autoclave indicator tape and autoclave for 30 min at 121° C. Allow to cool, place in garbage bag and discard in regular trash.

E. Verification

1. Total Coliform
 - Before inoculating refrigerated fermentation tube media incubate overnight and check for false positive gas bubbles.
 - For potable water samples, verify all presumptive colonies by using a sterile, disposable loop to pick from all presumptive colonies and verify as total coliform and/or *E. coli*.
- a. Using a sterile, disposable loop, transfer growth from all typical presumptive colonies to lauryl tryptose broth (LTB, HACH 21623-15), brilliant green lactose bile broth (BGLBB, HACH 322-15) and EC Medium w/MUG (HACH 22824-15). **If all 3 media are to be inoculated simultaneously the order shall be EC w/MUG – LTB - BGLB.** In the alternative, a single loop can be used to inoculate LTB-BGLBB (in that order), and a fresh loop used to inoculate the EC w/MUG.
- b. Incubate and observe for result according to Table B4 below.

Table B4

Medium	Incubation	Observation
Lauryl Tryptose Broth (LTB)	24-48 hours 35 ± 0.5° C	Turbidity/Gas
Brilliant Green Lactose Bile Broth (BGLBB)	24-48 hours 35 ± 0.5° C	Turbidity/Gas
EC Medium w/MUG	24 ± 2 hours 44.5 ± 0.2° C	Turbidity/Fluorescence

c. Interpretation

- Gas production w/in 24 hrs in both LTB and BGLBB tubes - confirmed for total coliform.
- No gas production w/in 24 hrs in LTB and BGLBB - incubate for an additional 24 hrs; if gas production occurs in both tubes - confirmed for total coliform.
- No gas production in either LTB or BGLBB following 48 hrs incubation – no total coliform present.
- Fluorescence w/in 24 hrs in EC w/MUG – positive for *E. coli*.
- If the LTB and BGLBB tubes are inoculated simultaneously and the LTB tube produces growth and gas and the BGLBB does not – inoculate a fresh BGLBB tube with inoculate from the positive LTB tube and incubate for 24 to 48 hours.

2. *Escherchia coli*

- Using sterile, disposable loops transfer growth from the center of at least 10 isolated typical colonies to EC Medium w/MUG (HACH 22824-15).
- Incubate for 24 ± 2 hrs in coliform water-bath at 44.5° C ± 0.2° C. Place all EC-MUG tubes in water-bath within 30 min after inoculation. Maintain a sufficient water depth in the water-bath to immerse tubes to upper level of medium.
- Examine each growth positive tube (turbid) for fluorescence by directing ultraviolet light (6-watt, 366 nm) at the tube from a distance of 5-6 inches in a darkened area.
- Fluorescence w/in 24 hrs in EC w/MUG – positive for *E. coli*.
- A positive control consisting of a known *E. coli* (MUG-positive) culture, a negative control consisting of a thermotolerant *Klebsiella pneumoniae* (MUG-negative) culture, and an uninoculated medium control will be included with each batch. This will aid in the interpretation of the results and avoid confusion of weak auto-fluorescence of the medium as a positive response.



3. Enterococci

- a. Using a sterile, disposable inoculating needle, transfer cells from the centers of at least 10 well isolated typical colonies into a brain heart infusion broth (BHIB) tube and onto a brain heart infusion agar (BHIA) slant.
- b. Incubate broth tubes for 24 hrs and slants for 48 hrs at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$.
- c. After 24 hr incubation, transfer a loopful of material from each BHIB tube to each of the following media:
 - a. BEA and incubate at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 48 hrs.
 - b. BHIB and incubate at $45^{\circ} \pm 0.5^{\circ}\text{C}$ for 48 hrs.
 - c. BHIB with 6.5% NaCl and incubate at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 48hrs.
- d. Observe for growth on all media.
- e. After 48hr incubation, apply a Gram stain to the growth from each BHIA slant.
- f. Gram positive cocci that grow and hydrolyze esculin on BEA (i.e. produce a black or brown precipitate) and grow in BHIB at $45^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and BHIB with 6.5% NaCl at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ are verified as enterococci.

F. Reporting

- For potable water testing report as presence or absence of total coliform and, if total coliform positive, report presence or absence of *E. coli*.
- For potable water, report all total coliform positives samples immediately to Laboratory Director and Chatham Water Department and request repeat samples.
- For all other sample types, with duplicate or greater filters per sample, report as number/100 ml using the following equation:

$$\text{Bacterial colonies/100 ml} = \frac{\text{Sum of colonies in all samples counted}}{\text{Sum of volumes (in ml) of all samples}} \times 100$$

G. Quality Control

Each new lot of media should be tested for proper performance.

Medium	Positive	Negative
mEndo Agar LES	<i>E. coli</i>	<i>Pseudomonas aeruginosa</i>
m-El	<i>Enterococcus faecalis</i>	<i>Streptococcus pyogenes</i>
m-TEC / Modified m-TEC	<i>E. coli</i>	<i>Klebsiella pneumonia</i>
Lauryl Tryptose Broth	<i>E. coli</i>	<i>Staphylococcus aureus</i>
Brilliant Green Lactose Bile Broth	<i>E. coli</i>	<i>Staphylococcus aureus</i>
EC w/MUG	<i>E. coli</i>	<i>Klebsiella pneumonia</i>



Green Infrastructure Stormwater Nitrogen BMP

Attachment B

BARNSTABLE, MA SAMPLING CHECKLISTS

Intersection of South Street and Pleasant Street

Barnstable, MA

Contents

1. Pre-Sampling Event Procedures Checklist	1
2. Sampling Procedures Checklist	2
3. Post-Sampling and Sample Preparation Procedures Checklist.....	3



1. PRE-SAMPLING EVENT PROCEDURES

_____ Check the future weather forecast for upcoming rain events >0.35-inches.

_____ Go to the Cape Cod Maritime Museum located at 135 South Street, Hyannis, MA or near the intersection of South Street and Pleasant Street in Barnstable, MA.

_____ Access sampling equipment through the external door at the rear of the site near the boat shed.

_____ Make sure Auto-Sampler bases are filled with uncapped sample bottles and are properly locked in place. Replace any if missing.

_____ Check the Auto-Sampler to be connected to the Signature Flow Meter and plugged into an outlet on the wall.

_____ Turn on the Signature Flow Meters and Auto-Samplers if not already on.

_____ Notify Ray Cody, EPA about the potential rainfall event and anticipated storm water samples:

Ray Cody, EPA Project Manager

cody.ray@epa.gov

617-918-1366



2. SAMPLING PROCEDURES

_____ Go to the Cape Cod Maritime Museum located at 135 South Street, Hyannis, MA or near the intersection of South Street and Pleasant Street in Barnstable, MA.

_____ Access sampling equipment through the external door at the rear of the site near the boat shed.

_____ Inspect the general condition of all of the equipment, tubing, casing, cables, and sensors; note any irregularities.

_____ Turn off the Auto-Sampler and cap all bottles in the carousel.

_____ Remove the full carousel for transport back to the office. **Note:** The inlet FWC Auto-Sampler may not fill all bottles; the outlet should always fill all 24 bottles with samples.

_____ Replace the full carousel with an empty carousel filled with uncapped sample bottles.

_____ Compare bottles within the full carousel and ensure the correct bottles were filled according to the Auto-Sampler readings; note any irregularities.

_____ Visually check to see if the sample volume appears to correspond with sample bottles according to the Auto-Sampler readings. Note any irregularities.

_____ Download data from the Signature Flow Meter and save on the USB; email the file(s) to: *Ray Cody, EPA*, cody.ray@epa.gov to determine FWC aliquots.

_____ Document the total number of samples collected successfully and unsuccessfully, the time of the first sample, last sample and stormwater runoff ending, excess sedimentation or debris accumulation and any maintenance activities to be followed-up.



3. POST-SAMPLING AND SAMPLE PREPARATION PROCEDURES

_____ Bring the full carousels to an appropriate work area. *Note:* Take care not to mix up carousels or alter the location of sample bottles within the carousel.

_____ **Discrete samples:** Remove each sample bottle and pour into a 1-liter EPA-provided sample bottle, taking careful note of the indexed number within the carousel and label each one. The Signature Flow Meter records the time, data, and ProPak number, indexed off of the inside of the Auto-sampler.

_____ **Flow-weighted samples:** Open the example flow-weighted composite spreadsheet. Follow the steps to determine the aliquots of each sample for compositing. Once each aliquot is determined, carefully composite water from the sample bottles into the EPA-provided bottle. *Note:* Two bottles will be needed, 1 for TN/TP and 1 for TSS, so each sample will have 2 aliquots of equal volume collected. Rinse and sanitize any instruments between aliquots.

_____ Label all EPA-provided sample bottles:

<site> <sample type> <analyte> <location> <date; time> <sample #> <time>

_____ Place all labeled sample bottles in a cooler with ice. Complete a Chain of Custody, place it in a plastic bag and tape it to the inside lid of the sample cooler.

_____ Ship or deliver the samples to EPA's National Exposure Research Laboratory (NERL) Monday through Thursday with the Chain of Custody:

EPA New England Regional Laboratory
11 Technology Drive
North Chelmsford, MA, 01863-2431

_____ Clean and decontaminate sample bottles. Rinse with tap water and laboratory detergent, then rinse again with tap water. Rinse thoroughly with DI water and allow to air dry completely.

_____ Once bottles are dry, reinsert into the carousel and cap bottles.



Green Infrastructure Stormwater Nitrogen BMP

Attachment C

BARNSTABLE, MA EQUIPMENT INVENTORY

Intersection of South Street and Pleasant Street

Barnstable, MA

Contents

1. Signature Flow Meter	2
2. ISCO 6712 Sampler.....	4
3. Calibrated Flume / Bubbler.....	6
4. TIENet 350 Area-Velocity Sensor	7
5. ISCO 674 Rain Gauge.....	8
6. Aqua TROLL 600 Multiparameter Sonde	10

1. Signature Flow Meter

The Signature Flow Meter functions as the 'base controller' for all other devices in the stormwater monitoring program including flow meters, the automatic sampler, and the rain gauge. This device ensures that all other equipment is connected, running, and logging data. When programmed, the Flow Meter will commence the storm-sampling event. The Flow Meter is also programmed to provide the sampling intervals to the Auto-Sampler. These intervals can be refined according to the storm's anticipated duration and intensity.

There are two (2) Signature Flow Meters at the Barnstable BMP site. This BMP site consists of a subsurface gravel wetland at an existing stormwater outfall in Barnstable, MA. Each Signature Flow Meter is set up for either the inlet or the outlet sampling locations. Refer to the Figure 7 at the end of Appendix A for the Barnstable site map and Signature Flow Meter locations.



Figure 1: Signature Flow Meter / Base Controller

Table 1: Signature Flow Meter Specifications	
Size (HxWxD)	<ul style="list-style-type: none"> 8.88 x 12.22 x 8.22 in. (with mounting bracket)
Data Storage	<ul style="list-style-type: none"> Non-volatile flash; retains stored data during program updates. Capacity: 8M Interval: 15 or 30 seconds; 1, 2, 5, 15, or 30 minutes; or 1, 2, 4, 12, or 24 hours Capacity: 180 days with 5 parameters logged at 1 minute intervals, reports once per day
Data Retrieval	<ul style="list-style-type: none"> USB drive Flowlink Software - with serial USB, remote cellular, or Ethernet

2. ISCO 6712 Sampler

The ISCO 6712 Sampler, or Auto-Sampler, is a portable machine that collects water samples automatically. The main components of the Auto-Sampler include the controller with a control panel (used to program the sampler settings), the adjustable distributor arm, the peristaltic pump/tubing, and the base containing sample bags. The base of the sampler has the capacity to hold twenty-four (24) ProPak sample bags. The Auto-Sampler is connected to the Signature Flow Meter which dictates when the sampling event starts.

There are two (2) Auto-Samplers at the Barnstable BMP site. This BMP site consists of a subsurface gravel wetland at an existing stormwater outfall in Barnstable, MA. Each Auto-Sampler is set up for either the inlet or the outlet sampling locations. The Auto-Samplers are programmed to take flow-weighted composite (FWC) grab samples. An additional fifth Auto-Sampler is located at the Barnstable inlet to collect discrete-time interval (DTI) grab samples. Refer to the Figure 7 for the Barnstable site map and Auto-Sampler locations.

After a sampling event, the samples must be labeled and sent to the laboratory for analysis with a completed chain of custody. For this monitoring program, Total Nitrogen (TN), Total Phosphorus (TP), and Total Suspended Solids (TSS) will be analyzed.



Figure 2: ISCO 6712 Sampler / Auto-Sampler

Table 2: Standard Programming Features	
Pacing	<ul style="list-style-type: none"> • Uniform Time Pacing: Sampling at regular time intervals. • Flow Pacing: Sampling at regular flow-volume intervals.
Distribution Methods	<ul style="list-style-type: none"> • Composite: Samples deposited in a single large bottle. • Sequential: only one sample placed in each bottle. • Samples per Bottle: Multiple samples placed in each bottle. • Bottles per Sample: One sample deposited into multiple bottles.

Refer to Table 3 below for the ISCO 6712 Sampler specifications.

Table 3: ISCO 6712 Sampler Specifications	
Size (Height x Diameter)	<ul style="list-style-type: none"> • 27 x 20 inches
Weight	<ul style="list-style-type: none"> • Dry, less battery - 32 lbs
Bottle configurations	<ul style="list-style-type: none"> • 24 - 1 Liter ProPak Disposable Sample Bags
Intake suction tubing	<ul style="list-style-type: none"> • Length: 3 to 99 feet • Inside dimension: 3/8 inch
Pump tubing life	<ul style="list-style-type: none"> • Typically 1,000,000 pump counts
Pump Maximum lift	<ul style="list-style-type: none"> • 28 feet
Pump Typical Repeatability	<ul style="list-style-type: none"> • ± 5 ml or $\pm 5\%$ of the average volume in a set
Pump Typical line velocity at Head height	<ul style="list-style-type: none"> • 3 ft. : 3.0 ft./s • 10 ft. : 2.9 ft./s • 15 ft. : 2.7 ft./s
Controller Weight	<ul style="list-style-type: none"> • 13 lbs.
Controller Size (HxWxD)	<ul style="list-style-type: none"> • 10.3 x 12.5 x 10 inches
Controller Operational temperature	<ul style="list-style-type: none"> • 32° to 120°F
Controller Number of composite samples	<ul style="list-style-type: none"> • Programmable from 1 to 999 samples
Sample frequency	<ul style="list-style-type: none"> • 1 minute to 99 hours 59 minutes, in 1 minute increments. Non-uniform times in minutes or clock times 1 to 9,999 flow pulses
Sampling modes	<ul style="list-style-type: none"> • Uniform time, non-uniform time, flow, event. (Flow mode is controlled by external flow meter pulses.)
Programmable sample volumes	<ul style="list-style-type: none"> • 10 to 9,990 ml in 1 ml increments
Sample retries	<ul style="list-style-type: none"> • If no sample is detected, up to 3 attempts; user selectable
Rinse cycles	<ul style="list-style-type: none"> • Automatic rinsing of suction line up to 3 rinses for each sample collection
Program storage	<ul style="list-style-type: none"> • 5 sampling programs
Sampling Stop/Resume	<ul style="list-style-type: none"> • Up to 24 real time/date sample stop/resume commands

3. Calibrated Flume / Bubbler

The Calibrated Flume and Bubbler acts as the low flow meter and outputs data to the Signature Flow Meter.

There are two (2) Calibrated Flumes/Bubbler flow measurement devices at the Barnstable BMP site. This BMP site consists of a subsurface gravel wetland at an existing stormwater outfall in Barnstable, MA. Each Calibrated Flume/Bubbler is set up for either the inlet or the outlet sampling locations. Refer to the Figure 7 at the end of Appendix A for the Barnstable site map and Calibrated Flume/Bubbler locations.

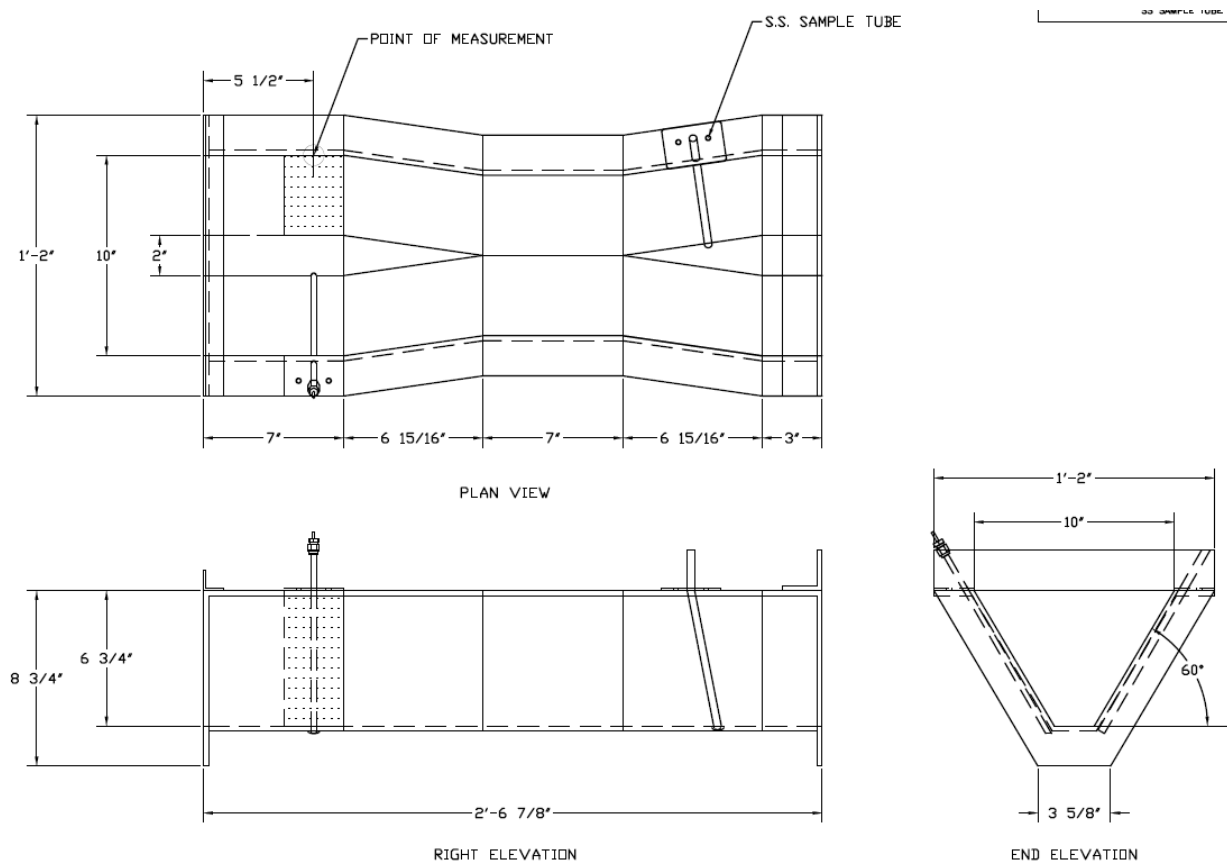


Figure 4: Calibrated Flume Dimensions

4. TIENet 350 Area-Velocity Sensor

The Area-Velocity (AV) Sensor acts as the high-flow meter and outputs data to the Signature Flow Meter. There are two (2) AV Sensors at the Barnstable BMP site. This BMP site consists of a subsurface gravel wetland at an existing stormwater outfall in Barnstable, MA. Each Area-Velocity Sensor is set up for either the inlet or the outlet sampling locations. Refer to the Figure 7 at the end of Appendix A for the Barnstable site map and AV Sensor locations.



Figure 5: Area-Velocity Sensor

Refer to Table 5 below for the specifications of the AV Sensor.

Table 5: Area-Velocity Sensor Specifications		
Sensor Dimensions	0.75 x 1.31 x 6.00 in	
Cable Diameter	0.402 in \pm 0.010 in	
Maximum Cabling from Signature Flow Meter	1,000 ft	
Typical Weight	w/ 5 m Cable: 1.95 lb w/ 10 m Cable: 3.70 lb w/ 23 m Cable: 6.84 lb	
Operation Temperature	32 to 158 °F	
Storage Temperature	-40 to 158 °F	
Level Range	0.033 to 10 ft	
Level Pressure Rating	5 PSI	
Maximum Submersible Depth	34.6 ft	
Level Accuracy	\pm 0.10% FS	
Typical Long Term Stability	\pm 0.023 ft/yr	
Compensated Temperature Range	32 to 158°F	
Velocity Direction	Bi-Directional (User selectable)	
Typical Minimum Depth for Velocity Measurement	1.0 in	
Velocity Range	-5 to +20 ft./s	
Velocity Accuracy	-5 to +5 ft./s:	\pm 0.1 ft./s (\pm 0.03 m/s)
	5 to 20 ft./s:	\pm 2% of reading

5. ISCO 674 Rain Gauge

The ISCO 674 Rain Gauge is a precise instrument which uses a tipping-bucket-design to measure rainfall. This Rain Gauge has an 8-inch diameter orifice and is calibrated to tip at either 0.01 inch or 0.1 mm of rainfall. The Rain Gauge is connected to the Signature Flow Meter and outputs rainfall data such as graphs and reports. The Signature Flow Meter relies on the Rain Gauge to activate sampling based on rainfall.

There are two (2) Rain Gauges at the Barnstable BMP site. This BMP site consists of a subsurface gravel wetland at an existing stormwater outfall in Barnstable, MA. Each Rain Gauge is set up for either the inlet or the outlet sampling locations. Refer to the Figure 7 at the end of Appendix A for the Barnstable site map and Rain Gauge locations.



Figure 6: ISCO 674 Rain Gauge

Refer to Table 6 below for a summary of features available for the ISCO 674 Rain Gauge.

Table 6: ISCO 674 Rain Gauge Features	
Standard Features	<ul style="list-style-type: none">• Screens cover all openings to prevent leaves, insects, and other debris from clogging the gauge.
Flow Meter Capabilities	<ul style="list-style-type: none">• Store rainfall data in internal memory for retrieval and analysis with Isco Flowlink Software• Activate sampling based on rainfall• Plot graphs and print reports of rainfall data on the flow meter's built in printer

Refer to Table 7 below for the ISCO 674 Rain Gauge specifications.

Table 7: ISCO 674 Rain Gauge Specifications	
Type	<ul style="list-style-type: none">• Tipping Bucket
Orifice Diameter	<ul style="list-style-type: none">• 8 in. (20 cm)
Sensitivity	<ul style="list-style-type: none">• English - 0.01 inch; Metric 0.1 mm
Accuracy	<ul style="list-style-type: none">• English - $\pm 1\%$ at 2 in/hour; +3%/-4% up to 5 in/hour• Metric - $\pm 1.5\%$ at 5 cm/hour; +3.5%/-9% up to 13 cm/hour
Capacity	<ul style="list-style-type: none">• English – 22 inches/hour• Metric – 38 cm/hour
Height	<ul style="list-style-type: none">• 13 in. (33 cm)
Diameter	<ul style="list-style-type: none">• 9.5 in. (24 cm) (at mounting base)
Weight	<ul style="list-style-type: none">• 10 lbs. (4.5 kg)
Operating Temperature	<ul style="list-style-type: none">• 32° to 140°F (0° to 60°C)
Storage Temperature	<ul style="list-style-type: none">• -40° to 140°F (-40° to 60°C)

6. Aqua TROLL 600 Multiparameter Sonde

The Aqua TROLL is a multiparameter sonde that collects accurate in-situ water quality data, including optical RDO, pH/ORP, turbidity, conductivity, temperature, and pressure. This instrument is corrosion-resistant, equipped with an antifouling system, and has the ability to maintain a battery life for 9+ months, minimizing maintenance needs. The Aqua TROLL is connected to the Signature Flow Meter and outputs varying water quality parameters during and in between storm events.

There are two (2) Aqua TROLL sondes deployed at the Barnstable BMP site. This BMP site consists of a subsurface gravel wetland at an existing stormwater outfall in Barnstable, MA. The Aqua TROLL sondes are set up near the inlet and outlet of the Barnstable BMP to record influent and effluent water quality characteristics. These sondes are deployed in standing water to ensure a sufficient moisture content is maintained to prevent the instruments' probes from damaging. Refer to the Figure 7 at the end of Appendix A for the Barnstable site map and Aqua TROLL sondes locations.


















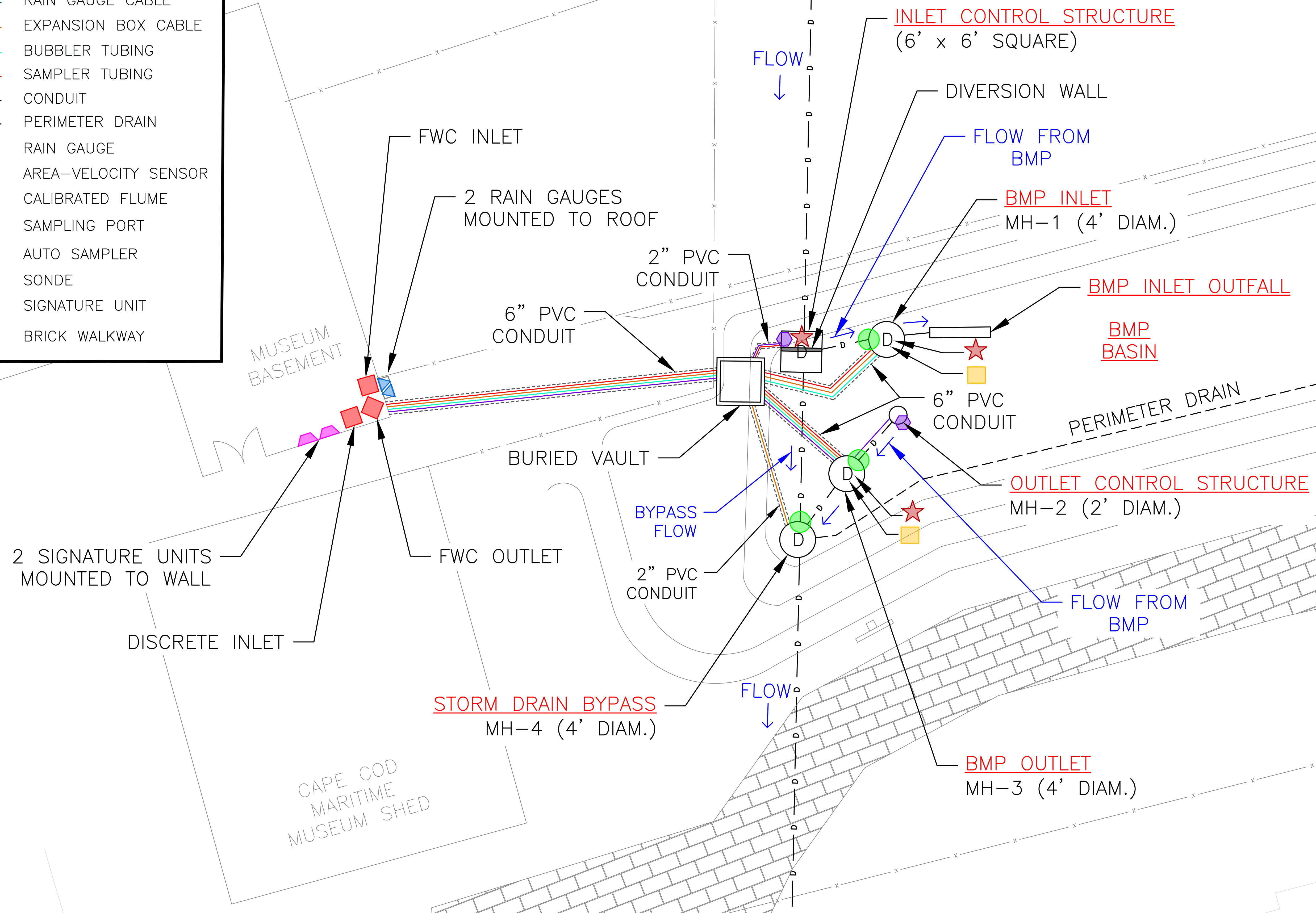
Figure 7: In-Situ Aqua TROLL Multiparameter Sonde

Table 8: In-Situ Aqua TROLL 600 Specifications	
Operating Temperature (nonfreezing)	<ul style="list-style-type: none">• 5 to 50° C (23 to 122° F) ISE: Ammonium & Nitrate 0 to 40° C ; Chloride 0 to 50° C
Storage Temperature	<ul style="list-style-type: none">• Components w/o fluid: -40° C to 65° C (non-freezing water); pH/ORP: -5° C to 65° C; Ammonium/Nitrate: 0 to 40 ° C; Chloride: 0 to 50° C
Dimensions	<ul style="list-style-type: none">• 4.7 cm (1.85 in.) OD x 60.2 cm (23.7 in.) (includes connector) With bail: 72.9 cm (28.7 in.)
Weight	<ul style="list-style-type: none">• 1.45 kg / 3.2 lbs (includes all sensors, batteries, and bail)
Wetted Materials	<ul style="list-style-type: none">• PC, PC alloy, Delrin™, Santoprene™, Inconel™, Viton™, Titanium, Platinum, Ceramic, Nylon
Environmental Rating	<ul style="list-style-type: none">• IP68 with all sensors and cable attached IP67 without the sensors, battery cover or cable attached
Max Pressure Rating	<ul style="list-style-type: none">• Up to 350 PSI

Output Options	<ul style="list-style-type: none"> • RS-485/MODBUS, SDI-12, Bluetooth®
Internal Memory 1 Micro SD Card 2	<ul style="list-style-type: none"> • 2 internal user-replaceable Alkaline D batteries <ul style="list-style-type: none"> ○ >6 months typical with wiping ○ >9 months typical with no wiping
Internal Power Battery Life 3	<ul style="list-style-type: none"> • -40° to 140°F (-40° to 60°C)
External Power Voltage	<ul style="list-style-type: none"> • 8-36 VDC (not required for normal operation) Sleep: 0.10 mA typical
External Power Current 4	<ul style="list-style-type: none"> • Measurement: 16 mA typical, 45 mA max
Reading Rates	<ul style="list-style-type: none"> • 1 reading every 2 seconds
Data Logging	<ul style="list-style-type: none"> • 50 logs (defined, scheduled to run, or stored)
Logging Modes	<ul style="list-style-type: none"> • Linear, Linear Average, Event
Hex Screw Driver	<ul style="list-style-type: none"> • 0.050, 1.3 mm
Communication Device	<ul style="list-style-type: none"> • TROLL Com or Wireless TROLL Com
Cable Options	<ul style="list-style-type: none"> • Vented or non-vented polyurethane or vented Tefzel®
LCD Display	<ul style="list-style-type: none"> • Integrated display shows status of sonde, sensor ports, data log, battery and connectivity
Software	<ul style="list-style-type: none"> • Android™: VuSitu through Google Play™, Windows®: Win-Situ 5, Data Services: HydroVu
Interface	<ul style="list-style-type: none"> • Android 4.4, requires Bluetooth 2.0; Win-Situ 5 Software
Certifications	<ul style="list-style-type: none"> • CE, FCC, WEEE, RoHS Compliant

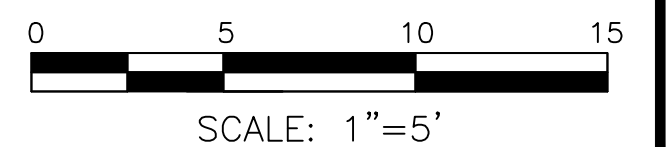
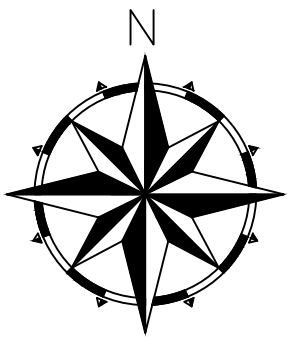
LEGEND

LINE STYLE	DESCRIPTION
	SONDE CABLE
	RAIN GAUGE CABLE
	EXPANSION BOX CABLE
	BUBBLER TUBING
	SAMPLER TUBING
	CONDUIT
	PERIMETER DRAIN
	RAIN GAUGE
	AREA-VELOCITY SENSOR
	CALIBRATED FLUME
	SAMPLING PORT
	AUTO SAMPLER
	SONDE
	SIGNATURE UNIT
	BRICK WALKWAY



GATEWAY MARINA BMP – SAMPLING SYSTEM SCHEMATIC

General Notes



No.	Revision/Issue	Date
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EPA Green Infrastructure Education and Outreach Project

GATEWAY MARINA BMP, GRAVEL BIORETENTION CELL

STORMWATER SAMPLING EQUIPMENT SCHEMATIC LAYOUT

South Street and Pleasant Street, Barnstable MA